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Clavulanic acid can be the component in amoxicillin-clavulanic acid responsible for immediate hypersensitivity reactions

To the Editor:

Immediate allergic reactions to penicillins usually appear within 1 hour after drug intake and are mediated by specific IgE antibodies.[1] Amoxicillin is the penicillin most frequently involved in sensitization, with the side chain playing a relevant role.[1] Amoxicillin is commercialized alone or combined to clavulanic acid (CLV). Although initial studies showed that CLV had a low immunogenic capacity,[2] allergic reactions to this compound may exist.[3,4]

We evaluated all patients (N = 307) seen at our allergy department for an allergic reaction after administration of amoxicillin-clavulanic acid (AX-CLV) over 3 years (2006-2008). Those finally diagnosed as having had an immediate allergic reaction and with a positive skin test were included in this study. Thirty cases with good tolerance to AX-CLV were used as controls. The study was approved by the institutional review board, and informed consent was obtained.

Skin testing was performed as described[5] by using benzylpenicilloyl-polylysine (PPL; 5×10^{-5} M), minor determinant mixture (2×10^{-2} M), and CLV (20 mg/mL), all provided by Diater (Madrid, Spain), and amoxicillin (GlaxoSmithKline Beecham, Madrid, Spain; 20 mg/mL). In those cases with a positive skin test to CLV, AX-CLV (GlaxoSmithKline Beecham) was also tested (20 mg/mL amoxicillin and 4 mg/mL CLV).

Single-blind, placebo-controlled drug provocation testing (DPT) was done by using benzylpenicillin, amoxicillin, and AX-CLV, as reported.[5] This approach enabled us to classify patients as allergic to benzylpenicillin, amoxicillin, or CLV.

Basophil activation testing (BAT) was done as described,[6] with benzylpenicillin, amoxicillin, AX-CLV, and CLV at 4 different concentrations (this article's Fig E1 in the Online Repository at www.jacionline.org shows the dose-response curve). Results were considered positive when the stimulation index (SI), calculated as a ratio between the percentage of activated basophils with the different haptens and the negative control, was ≥2 to at least one of the concentrations mentioned.

To confirm that the drug-induced basophil activation was IgE-mediated, we analyzed the wortmannin inhibitory effect at different concentrations (5, 1, 0.1 μmol/L) with benzylpenicillin, amoxicillin, AX-CLV, CLV, and the positive controls (chemotactic peptide N-Formyl-Met-Leu-Phe [fMLP] and anti-IgE).[7] We also followed up the BAT-positive patients at 6-month intervals over 1 year to evaluate the specific IgE clearance.

Comparisons for quantitative variables without a normal distribution were done by the Kruskal-Wallis tests using SPSS (15.0; IBM, Chicago, Ill).

Of the initial group of 307 patients evaluated with a reaction attributed to AX-CLV, 31 refused or did not complete the study.

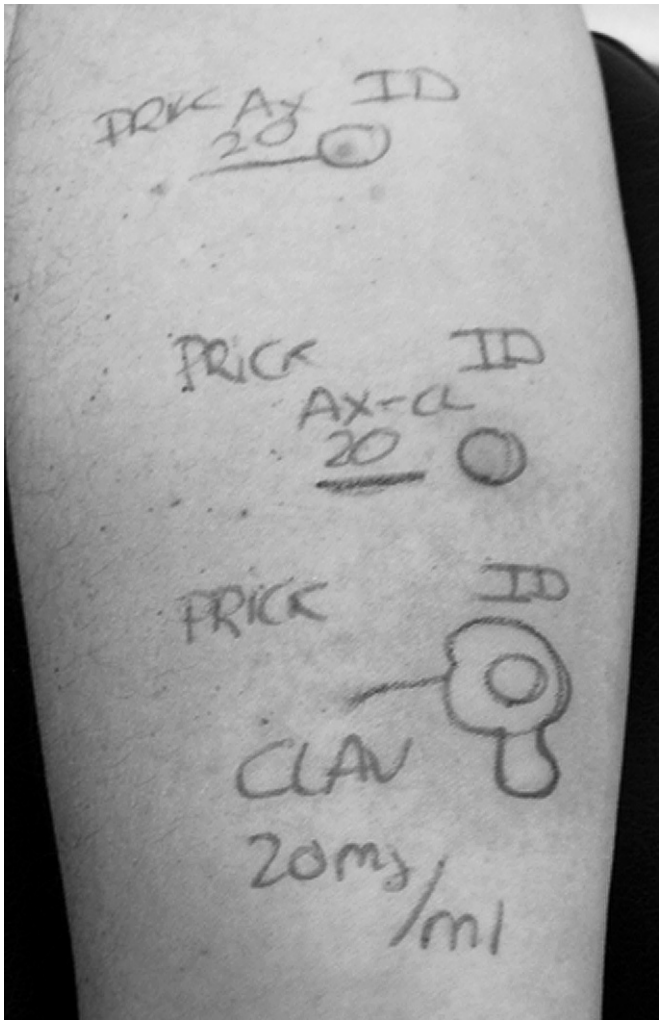

FIG 1. Skin test results using amoxicillin (20 mg/mL), amoxicillin-clavulanic acid (20/4 mg/mL), and clavulanic acid (20 mg/mL) in patient 55C. On the *left* are shown prick results (all negative) and on the *right* intradermal results (only positive to clavulanic acid).

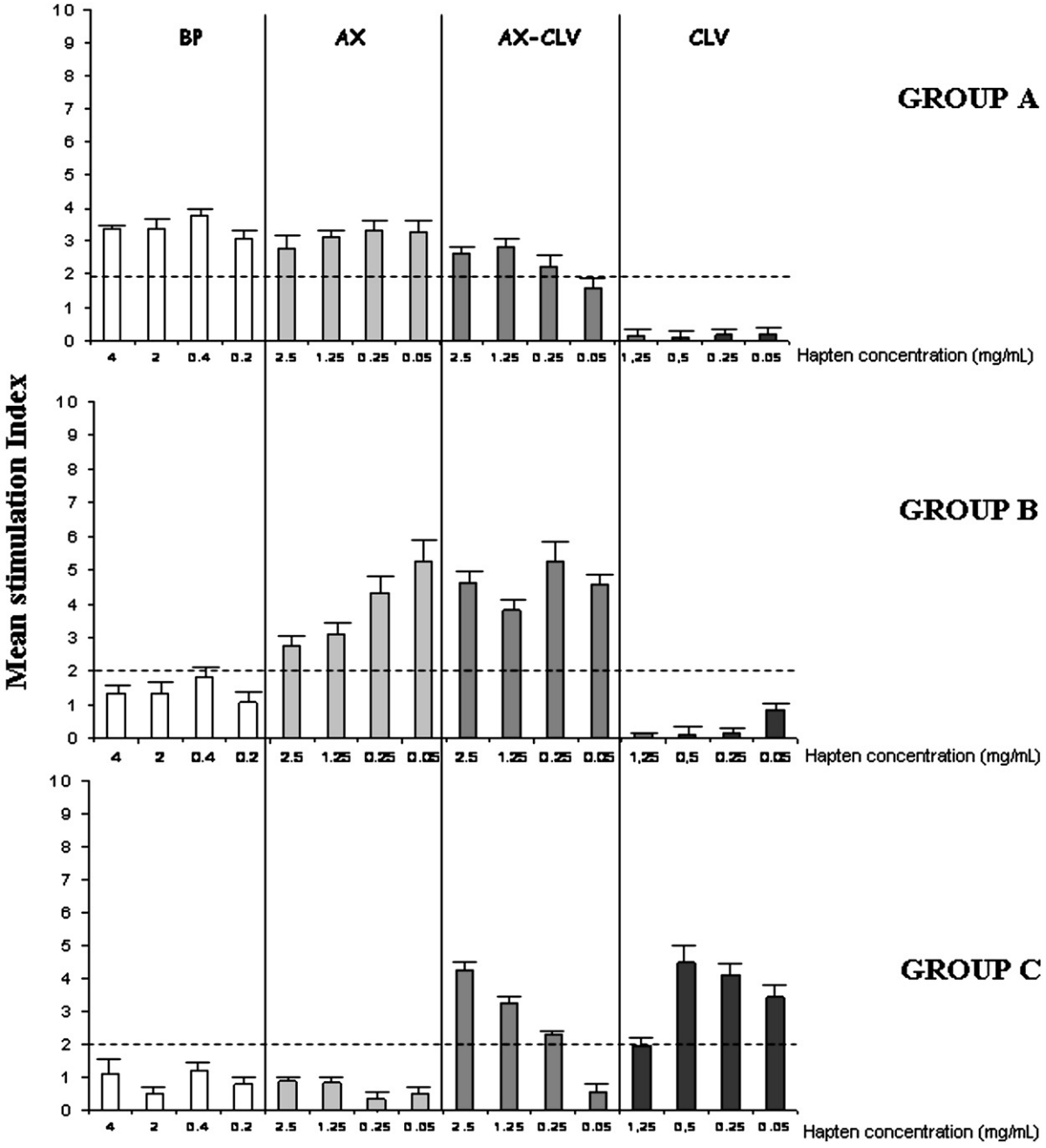

FIG 2. Mean and SD of SI from BAT in positive cases in the 3 groups, with benzylpenicillin *(BP)*, amoxicillin *(AX)*, AX-CLV, and CLV at different concentrations (mg/mL). The AX-CLV concentration expresses the AX concentration in the combination.

Of the remaining 276, fifty-five (19.9%) had a positive skin test result to different penicillin determinants. Those with negative skin tests (N = 221) underwent DPT, with the following results: 199 had good tolerance to benzylpenicillin, amoxicillin, and AX-CLV and were considered not to have allergy; 15 had good tolerance to benzylpenicillin, developed an immediate reaction to amoxicillin, and were considered allergic to amoxicillin; and 7 had good tolerance to benzylpenicillin and amoxicillin, developed an immediate reaction to AX-CLV, and were considered allergic to CLV.

Those with positive skin tests (N = 55) were subjected to further analysis and classified in 3 groups, according to skin test results and DPT: group A (N = 5; 9%), patients with a positive skin test result to PPL or minor determinant mixture; group B (N = 34; 62%), patients with a negative skin test result to PPL and minor determinant mixture, good tolerance to benzylpenicillin by DPT, and a positive skin test to amoxicillin; and group C (N = 16; 29%), patients with a negative skin test result to PPL, minor determinant mixture and amoxicillin, good tolerance by DPT to benzylpenicillin and amoxicillin, and a positive skin test to CLV.

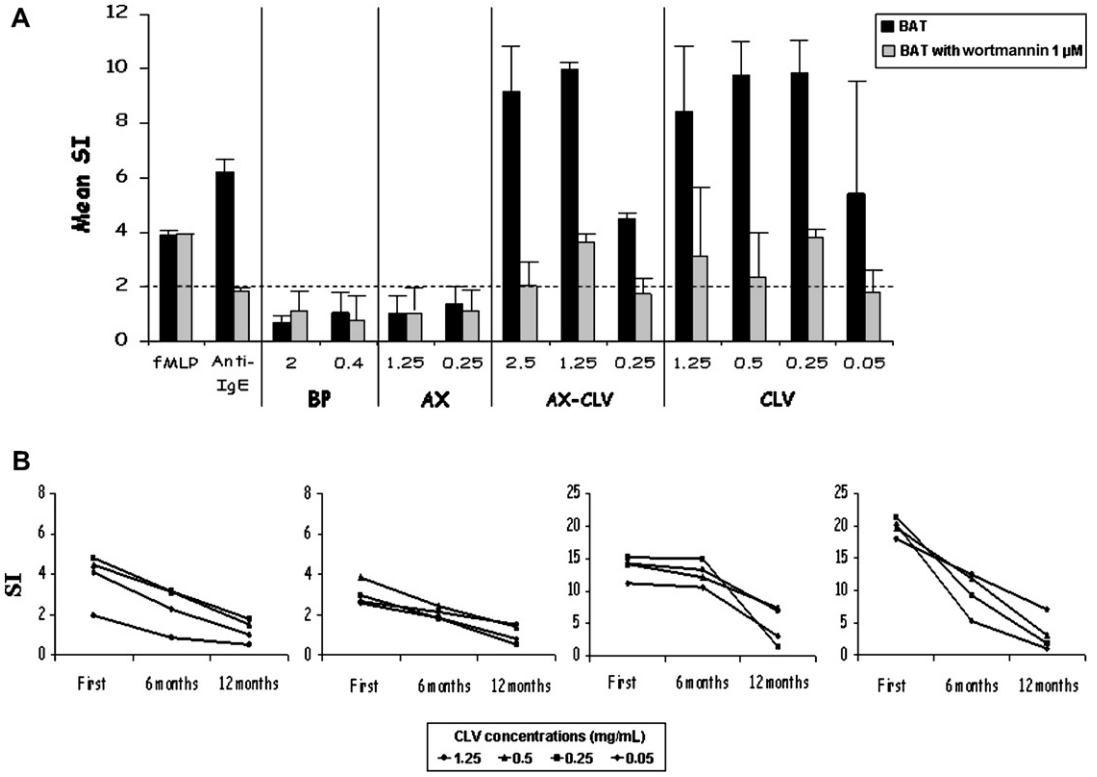

FIG 3. A, Wortmannin inhibitory effect in basophil activation under different conditions: positive controls (fMLP and anti-IgE), benzylpenicillin *(BP)*, amoxicillin *(AX)*, AX-CLV, and CLV at different concentrations (mg/mL). These data show the mean SI from 4 patients of group C. **B,** BAT results at different CLV concentrations (1.25, 0.5, 0.25, and 0.05 mg/mL) during a follow-up period of 12 months. These figures represent individual data from 4 patients of group C.

In group C, skin testing with AX-CLV was also performed and was positive in 10 cases (Fig 1; see this article's Table E1 in the Online Repository at www.jacionline.org). Comparisons between groups showed significant differences for age ($P < .005$), with group A the oldest and group C the youngest.

The BAT was done in all 55 patients and was positive in 29 (52.7%): 3 (60%) in group A, 18 (52.9%) in group B, and 8 (50%) in group C. The mean SI values in the positive cases from each group are shown in Fig 2. The BAT was also done in the control group, showing 90% specificity. We observed that the phosphatidylinositol 3-kinase (PI3K) inhibitor, wortmannin, induced BAT inhibition when stimulated with anti-IgE and with haptens, especially in those patients with positive BAT results, but not when fMLP was used as the basophil stimulator (Fig 3, *A*). Moreover, the BAT decreased, even becoming negative, in those cases with low BAT positivity (Fig 3, *B*).

Although CLV has a β-lactam ring able to bind covalently to proteins, it has important chemical differences with penicillin antibiotics: it lacks the side chain, and the thiazolidine ring has been substituted by an oxazolidine. These differences may contribute to the generation of allergenic determinants with little or no cross-reactivity with those generated by benzylpenicillin or amoxicillin. With the increased consumption of AX-CLV, more and more patients have appeared with immediate reactions to this combination, with most studies indicating that amoxicillin was the inducer.[1,8] Evidence about whether CLV could be the culprit drug is so far limited to just a few case reports.[3,4]

In our study, 20% of the patients were confirmed as having had an immediate allergic reaction to AX-CLV with a positive skin test to 1 or more of the reagents used. We found that just 9% (group A) recognized benzylpenicillin determinants, and an important percentage (62%) recognized amoxicillin determinants. This indicates that amoxicillin is still the most frequently involved penicillin in inducing sensitization.[1,8] Notably, whereas 30% of patients had a positive skin test result to CLV and had good tolerance to amoxicillin, only 18% were detected by AX-CLV. Because skin test sensitivity to β-lactam determinants is concentration-dependent, we believe these differences are related to the CLV concentration used (20 mg/mL when used alone vs 4 mg/mL when AX-CLV was used). Because AX-CLV has so far been the only drug available for skin testing, this may explain why more reactions with a positive skin test result to CLV have not been reported.

The pattern of responses observed in the BAT studies confirmed the existence of these 3 groups, with patients recognizing penicillin determinants, patients recognizing amoxicillin-specific determinants, and patients specifically recognizing CLV determinants. Because basophil activation could be induced by non–IgE-mediated mechanisms, we tried to confirm the involvement of specific IgE in our cases by using a PI3K inhibitor, wortmannin, under different basophil stimulation conditions. PI3K is one of the important kinases activated by FcεRI receptor cross-linking and is involved in regulating histamine release.[9] Although PI3K inhibition with wortmannin can also inhibit IL-3 and

GM-CSF–induced activation of basophils, these cytokines do not induce activation, nor do they induce CD63. In addition, it is unlikely that IL-3, GM-CSF, or even monocyte chemoattractant protein MCP 1 activities were present in our drug solutions. Therefore, we consider that our results with wortmannin and the negativization over time support an IgE-mediated response.

Our results indicate that immediate selective reactions to CLV do exist and account for around 30% of immediate allergic reactions in cases that are positive after taking the combination of AX-CLV. We recommend using CLV in the diagnostic evaluation in cases of immediate reactions in which the association AX-CLV is incriminated when conventional skin testing with benzylpenicillin and amoxicillin determinants proves negative.

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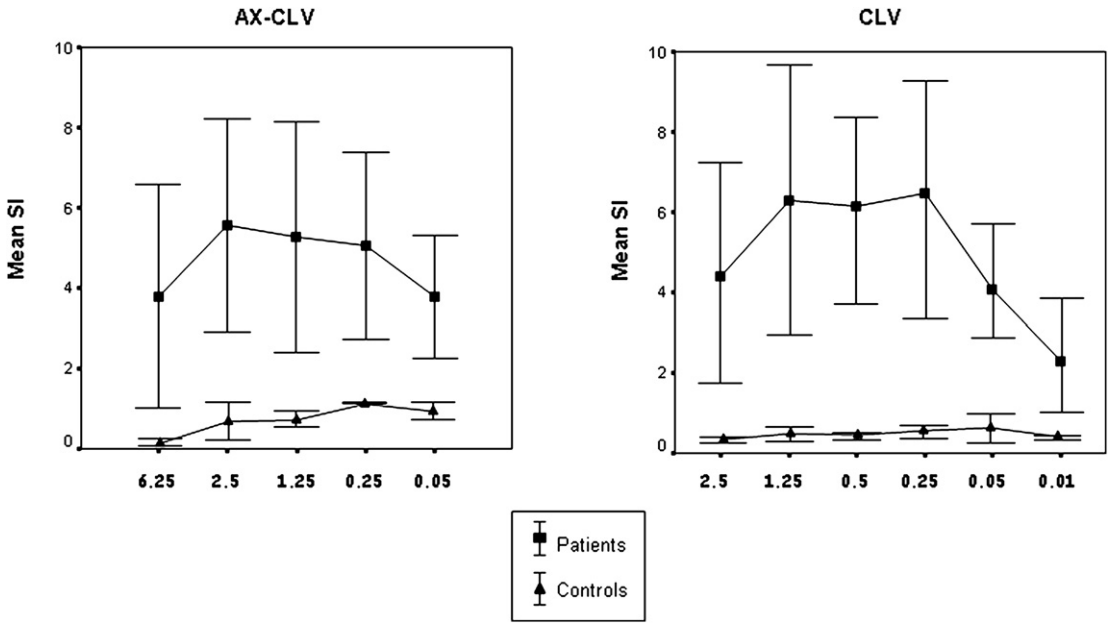

FIG E1. Dose -response curve for positive patients (N = 8) and controls (N = 8), responders to AX-CLV **(A)** or CLV **(B)**. Results are expressed as the SIs (means ± SEMs).

TABLE E1. Clinical characteristics and skin test and drug provocation test results of the study patients

					Skin test (wheal increase in mm)					DPT	
Patient no.	Age (y)	Sex	Interval (mo)	Reaction	PPL	MDM	AX	CLV	AX-CLV	BP	AX
1A	61	F	6	Urticaria	ID (+) (3 × 3)	(–)	(–)	(–)	ND	ND	ND
2A	47	F	5	Urticaria	(–)	ID (+) (3 × 4)	ID (+) (5 × 6)	(–)	ND	ND	ND
3A	63	M	1	Anaphylaxis	(–)	P (+) (4 × 3)	P (+) (4 × 4)	(–)	ND	ND	ND
4A	59	F	1	Anaphylaxis	ID (+) (5 × 7)	ID (+) (4 × 6)	P (+) (5 × 6)	(–)	ND	ND	ND
5A	48	M	18	Anaphylaxis	(–)	P (+) (4 × 5)	P (+) (4 × 3)	(–)	ND	ND	ND
6B	49	F	4	Anaphylaxis	(–)	(–)	ID (+) (5 × 6)	(–)	ND	(–)	ND
7B	20	F	2	Urticaria	(–)	(–)	ID (+) (3 × 4)	(–)	ND	(–)	ND
8B	37	M	9	Urticaria	(–)	(–)	ID (+) (3 × 3)	(–)	ND	(–)	ND
9B	55	F	5	Anaphylaxis	(–)	(–)	ID (+) (4 × 6)	(–)	ND	(–)	ND
10B	41	F	7	Urticaria	(–)	(–)	ID (+) (3 × 3)	(–)	ND	(–)	ND
11B	42	M	4	Anaphylaxis	(–)	(–)	ID (+) (5 × 7)	(–)	ND	(–)	ND
12B	52	F	6	Anaphylaxis	(–)	(–)	ID (+) (3 × 4)	(–)	ND	(–)	ND
13B	47	F	5	Anaphylaxis	(–)	(–)	P (+) (4 × 5)	(–)	ND	(–)	ND
14B	30	M	24	Anaphylaxis	(–)	(–)	ID (+) (5 × 7)	(–)	ND	(–)	ND
15B	46	M	46	Anaphylaxis	(–)	(–)	ID (+) (3 × 4)	(–)	ND	(–)	ND
16B	19	F	12	Anaphylaxis	(–)	(–)	ID (+) (4 × 6)	(–)	ND	(–)	ND
17B	57	M	2	Anaphylaxis	(–)	(–)	P (+) (4 × 5)	(–)	ND	(–)	ND
18B	45	F	4	Urticaria	(–)	(–)	ID (+) (3 × 3)	(–)	ND	(–)	ND
19B	46	M	6	Anaphylaxis	(–)	(–)	ID (+) (5 × 6)	(–)	ND	(–)	ND
20B	31	M	5	Anaphylaxis	(–)	(–)	ID (+) (3 × 4)	(–)	ND	(–)	ND
21B	37	M	4	Anaphylaxis	(–)	(–)	ID (+) (5 × 6)	(–)	ND	(–)	ND
22B	54	M	2	Anaphylaxis	(–)	(–)	ID (+) (5 × 5)	(–)	ND	(–)	ND
23B	38	F	1	Anaphylaxis	(–)	(–)	P (+) (4 × 5)	(–)	ND	(–)	ND
24B	67	M	2	Anaphylaxis	(–)	(–)	ID (+) (3 × 4)	(–)	ND	(–)	ND
25B	46	M	1	Anaphylaxis	(–)	(–)	P (+) (4 × 6)	(–)	ND	(–)	ND
26B	60	F	1	Anaphylaxis	(–)	(–)	P (+) (4 × 5)	(–)	ND	(–)	ND
27B	60	M	1	Anaphylaxis	(–)	(–)	ID (+) (5 × 6)	(–)	ND	(–)	ND
28B	18	M	2	Anaphylaxis	(–)	(–)	ID (+) (3 × 4)	(–)	ND	(–)	ND
29B	47	F	4	Anaphylaxis	(–)	(–)	P (+) (4 × 4)	(–)	ND	(–)	ND
30B	53	M	2	Urticaria	(–)	(–)	ID (+) (5 × 6)	(–)	ND	(–)	ND
31B	36	F	5	Anaphylaxis	(–)	(–)	ID (+) (3 × 4)	(–)	ND	(–)	ND
32B	41	M	4	Anaphylaxis	(–)	(–)	ID (+) (5 × 6)	(–)	ND	(–)	ND
33B	35	M	6	Anaphylaxis	(–)	(–)	ID (+) (4 × 5)	(–)	ND	(–)	ND
34B	44	F	12	Anaphylaxis	(–)	(–)	ID (+) (3 × 4)	(–)	ND	(–)	ND
35B	47	M	1	Anaphylaxis	(–)	(–)	P (+) (4 × 5)	(–)	ND	(–)	ND
36B	59	M	1	Anaphylaxis	(–)	(–)	ID (+) (5 × 5)	(–)	ND	(–)	ND
37B	55	M	1	Anaphylaxis	(–)	(–)	P (+) (3 × 4)	(–)	ND	(–)	ND
38B	48	M	2	Anaphylaxis	(–)	(–)	P (+) (4 × 4)	(–)	ND	(–)	ND
39B	57	M	1	Anaphylaxis	(–)	(–)	ID (+) (3 × 3)	(–)	ND	(–)	ND
40C	42	F	22	Anaphylaxis	(–)	(–)	(–)	ID (+) (3 × 3)	ID (+) (3 × 4)	(–)	(–)
41C	26	M	5	Anaphylaxis	(–)	(–)	(–)	ID (+) (3 × 5)	ID (+) (3 × 4)	(–)	(–)
42C	59	F	4	Anaphylaxis	(–)	(–)	(–)	ID (+) (5 × 5)	ID (+) (3 × 4)	(–)	(–)
43C	18	M	2	Urticaria	(–)	(–)	(–)	P (+) (5 × 6)	ID (+) (5 × 6)	(–)	(–)
44C	35	M	2	Anaphylaxis	(–)	(–)	(–)	ID (+) (4 × 6)	ID (+) (4 × 4)	(–)	(–)
45C	56	F	12	Urticaria	(–)	(–)	(–)	ID (+) (5 × 6)	(–)	(–)	(–)
46C	42	M	1	Anaphylaxis	(–)	(–)	(–)	P (+) (4 × 4)	P (+) (4 × 3)	(–)	(–)
47C	30	M	11	Anaphylaxis	(–)	(–)	(–)	ID (+) (3 × 4)	(–)	(–)	(–)
48C	30	M	3	Anaphylaxis	(–)	(–)	(–)	ID (+) (5 × 4)	ID (+) (3 × 4)	(–)	(–)
49C	18	M	1	Anaphylaxis	(–)	(–)	(–)	ID (+) (4 × 6)	ID (+) (5 × 5)	(–)	(–)
50C	38	F	10	Anaphylaxis	(–)	(–)	(–)	ID (+) (4 × 4)	(–)	(–)	(–)
51C	47	F	4	Urticaria	(–)	(–)	(–)	ID (+) (5 × 5)	(–)	(–)	(–)
52C	49	F	1	Anaphylaxis	(–)	(–)	(–)	P (+) (5 × 4)	P (+) (3 × 4)	(–)	(–)
53C	28	M	7	Anaphylaxis	(–)	(–)	(–)	ID (+) (6 × 4)	ID (+) (3 × 4)	(–)	(–)
54C	34	M	10	Anaphylaxis	(–)	(–)	(–)	ID (+) (5 × 7)	(–)	(–)	(–)
55C	31	F	15	Anaphylaxis	(–)	(–)	(–)	ID (+) (4 × 7)	(–)	(–)	(–)

AX, Amoxicillin; *BP*, benzylpenicillin; *F*, female; *ID*, intradermal; *M*, male; *MDM*, minor determinant mixture; *ND*, not done; *P*, prick.

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Selective allergic reactions to clavulanic acid: A report of 9 cases

To the Editor:

Clavulanic acid is a β-lactam antibiotic with weak antibacterial activity but is a potent inhibitor of β-lactamases. In clinical practice clavulanic acid is available as a combination product with amoxicillin.[1]

Skin prick and intradermal tests are the main diagnostic methods used to confirm clinical hypersensitivity after immediate allergic reactions to β-lactam antibiotics.[2] Prior studies have demonstrated that quantification of *in vitro* basophil activation carried out by means of flow cytometry can be reliable for measuring IgE-dependent, allergen-specific responses in patients who are allergic to β-lactams,[3] as well as to clavulanic acid.[2]

We describe 9 cases of allergic reactions to clavulanic acid that were diagnosed in our department between 2004 and 2009. We used skin test results and performed basophil activation tests to detect the presence of specific IgE.

Nine patients, 4 women and 5 men, who were 19 to 58 years of age (mean age, 42.4 years) were referred to our allergy department with adverse reactions to amoxicillin–clavulanic acid. Clinical symptoms appeared immediately (within 60 minutes of intake) in 5 of the patients and after 2 hours in the other 4 patients. The symptoms reported were maculopapular rash in 3 patients, urticaria in 3 patients, urticaria accompanied by angioedema in 2 patients, and anaphylaxis (generalized erythema with pruritus, hives, and dyspnea) in 1 patient. In 3 patients the reaction also involved other drugs. In 2 patients paracetamol and amoxicillin–clavulanic acid were involved, and in the third patient paracetamol, acetylcysteine, and amoxicillin–clavulanic acid were the implicated drugs. We performed skin tests and single-blind oral challenges with the other implicated drugs with negative results, and therefore these drugs were ruled out as casual. None of these subjects reported prior adverse drug reactions. All patients had previously received amoxicillin–clavulanic acid without adverse events. The characteristics of the reactions and patients' ages are shown in Table I. The patients consented in writing before skin tests, and controlled challenge tests were performed, although institutional review board approval was not obtained.

TABLE I. Characteristics of the reactions

Patient no.	Age (y)	Year of reaction	Latency	Symptoms
1	19	2004	2 d	Maculopapular rash
2	50	2004	4 d	Urticaria
3	52	2008	45 min	Maculopapular rash
4	33	2008	2 h	Urticaria and angioedema
5	43	2008	45 min	Anaphylaxis
6	40	2008	45 min	Urticaria and angioedema
7	58	2008	30 min	Maculopapular rash
8	41	2009	30 min	Urticaria
9	46	2009	4 h	Urticaria

Results of skin prick and intradermal tests with benzylpenicilloyl poly-L-lysine as the major determinant at a concentration of 0.04 mg/mL and a minor determinant mixture (benzylpenicillin sodium, benzylpeniciloic acid, and sodium benzylpenicilloate) at 0.5 mg/mL were negative in all patients (Diater SA, Madrid, Spain). In addition, results of skin prick and intradermal tests with benzylpenicillin (10,000 IU/mL; Normon SA, Madrid, Spain), amoxicillin (20 and 25 mg/mL; GlaxoSmithKline SA, Madrid, Spain), ampicillin (20 mg/mL, Normon SA), cefuroxime (2 mg/mL, GlaxoSmithKline SA), and ceftazidime (2 mg/mL; Combino Pharm SL, Barcelona, Spain) were all negative. Results of skin prick tests with amoxicillin–clavulanic acid (20 mg/mL, GlaxoSmithKline SA) were also negative in the 9 patients. Results of intradermal tests with amoxicillin–clavulanic acid at 20 mg/mL were positive in all patients except for patient 2. Skin tests with amoxicillin–clavulanic acid were performed with the intravenous suspension of this drug. Control subjects were subjects with a history of an adverse reaction to β-lactams that was not confirmed (negative skin test and oral challenge results). Specific IgE antibodies against penicillin V, penicillin G, amoxicillin, and ampicillin (CAP-FEIA; Phadia, Uppsala, Sweden) were less than 0.35 UI/mL in all patients.

Because skin test results and specific IgE levels for amoxicillin were negative and skin test results to amoxicillin–clavulanic acid were positive in 8 of 9 patients, single-blind oral challenges were performed with amoxicillin. In addition, patient 2, who had a negative skin test result with amoxicillin–clavulanic acid, underwent a single-blind oral challenge with amoxicillin–clavulanic acid because this was the drug suspected in the original reaction referred by the patient. Oral challenges were performed with amoxicillin (125, 250, and 500 mg) and amoxicillin–clavulanic acid (125/31.25, 250/62.5, and 500/125 mg), increasing doses at 1-hour intervals. In nonimmediate reactions (patients 1, 2, 4, and 9) the therapeutic dose was subsequently taken at home every

TABLE II. Results of the allergy study

Case	Skin tests	Specific IgE	Oral challenge	Clavulanic acid skin tests	Basophil activation test
1	IDR AM-CL + IDR AM −	<0.35 kU/L	AM −	Negative	Basal stimulation
2	IDR AM-CL − IDR AM −	<0.35 kU/L	AM CL + AM −	Negative	CL+Syloid +
3	IDR AM-CL + IDR AM −	<0.35 kU/L	AM −	Positive	Basal stimulation
4	IDR AM-CL + IDR AM −	<0.35 kU/L	AM −	Positive	CL+Syloid +
5	IDR AM-CL + IDR AM −	<0.35 kU/L	AM −	Positive	CL+Avicel +
6	IDR AM-CL + IDR AM −	<0.35 kU/L	AM −	Positive	Basal stimulation
7	IDR AM-CL + IDR AM −	<0.35 kU/L	AM −	Positive	CL+Syloid +
8	IDR AM-CL + IDR AM −	<0.35 kU/L	AM −	Positive	Not assessable
9	IDR AM-CL + IDR AM −	<0.35 kU/L	AM −	Positive	Negative

AM, Amoxicillin; *AM-CL*, amoxicillin-clavulanic acid; *CL+Syloid*, clavulanic acid and Syloid; *CL+Avicel*, clavulanic acid and Avicel; *IDR*, intradermal test.

8 hours for 7 days. The result of a single-blind oral challenge with amoxicillin–clavulanic acid in patient 2 was positive, with a delayed reaction. Twenty-four hours after the oral challenge with amoxicillin–clavulanic acid, (cumulative dose of 1,875/468.75 mg), the patient experienced a generalized maculopapular rash that resolved over 48 hours with antihistamines and corticosteroids. Results of single-blind oral challenges with amoxicillin in all patients, including patient 2, were negative.

In 2009, we obtained for the first time purified clavulanic acid (Normon SA) for patient testing. Clavulanic acid is inherently unstable in solution, requiring the use of excipients, either Avicel (cellulose) or Syloid (silica). Commercially, Avicel (Normon SA) is used in oral tablets, and Syloid (Normon SA) is used for oral suspensions. For skin testing, clavulanic acid was mixed one to one with one of the excipients, and concentrations of 10 mg/mL (both of clavulanic acid and excipient) were used for skin prick testing, with concentrations of 0.1 and 1 mg/mL used for intradermal testing. We contacted all of the patients previously given diagnoses of suspected clavulanic acid sensitivity, and all agreed to testing. All subjects had negative skin prick test responses at 10 mg/mL clavulanic acid and excipient and negative intradermal test results with 0.1 mg/mL. However, 7 of 9 patients had positive intradermal test results with 1 mg/mL clavulanic acid (patients 1 and 2 had negative results). Skin prick and intradermal test results with clavulanic acid and excipients were negative in 10 control subjects. These subjects were selected from our practice based on a history of a prior adverse event when treated with amoxicillin–clavulanic acid but who had subsequently tolerated a challenge with amoxicillin–clavulanic acid.

As previously described,[3] flow cytometric analysis of CD63 expression by basophils was performed after *in vitro* allergen-specific stimulation with clavulanic acid (with Syloid and with Avicel) at concentrations of both 0.05 and 0.5 mg/mL. Excipient and clavulanic acid concentrations were the same as used for skin testing. Five subjects with prior negative challenge results to amoxicillin–clavulanic acid served as negative control subjects. The basophil activation test results were positive in 4 patients at both concentrations, 3 with clavulanic acid with Avicel and 1 with clavulanic acid with Syloid. Three patients had a basal stimulation of basophils, probably because of nonspecific stimulation; therefore the results were not assessable. One patient had negative results, and another was not assessable because of a low number of basophils. All control subjects had negative results in specific basophil activation with both concentrations of clavulanic acid and both excipients. The results of allergy study are shown in Table II.

Allergic reactions to β-lactam antibiotics are the most frequent cause of adverse drug reactions caused by IgE-mediated mechanisms.[4] Clavulanic acid has been associated with very few allergic reactions, suggesting a low allergenic potential.[5]

The diagnosis of clavulanic acid hypersensitivity is complicated by limited availability of clavulanic acid in a stable solution for skin or *in vitro* testing.[5] We obtained clavulanic acid alone and performed skin prick and intradermal tests with positive results in 7 of 9 patients with suspected allergy to clavulanic acid and in none of 10 subjects who tolerated amoxicillin–clavulanic acid. Negative skin test results with amoxicillin–clavulanic acid in 2 patients might be explained by a 5-year time period between the original adverse reaction and skin tests with amoxicillin–clavulanic acid and the purified clavulanic acid. Skin test reactivity to penicillin decreases annually after an allergic reaction to the antibiotic. Therefore 73% continued to have a positive response within 1 year of the reaction, 57% between 1 and 10 years, and 22% at 10 years or more after the reaction.[6] Therefore 2 of 9 patients might have lost skin test reactivity after the passage of 5 years. This point is speculative.

Despite the extensive use of amoxicillin–clavulanic acid, few reports of a drug allergy to clavulanic acid have been reported.[1,2,5,7-12] In the first article Fernández-Rivas et al[1] described 2 cases of immediate adverse reactions to amoxicillin–clavulanic acid. Skin test results with clavulanic acid were positive in both patients. Cahen and Wüthrich[9] performed scratch tests and a skin prick test with clavulanic acid, both with positive results. González de Olano et al[7] reported a patient with urticaria after receiving amoxicillin–clavulanic acid. The diagnosis resulted from a positive oral challenge result with amoxicillin–clavulanic acid and a negative challenge result with amoxicillin.

There are 3 reports of delayed reaction to clavulanic acid.[8,10,12] Kamphof et al[10] and Hoon Kim et al[8] performed patch tests with clavulanic acid, and positive results were obtained in both cases.

Longo et al[2] described 2 patients with urticaria and angioedema after oral amoxicillin–clavulanic acid. The basophil activation test and sulphidoleukotriene release by basophils revealed a positive response with clavulanic acid.

During the last 5 years, we have observed an apparent increase in patients with suspected sensitization to clavulanic acid, probably because of the increasing use of amoxicillin associated with this drug over this period. Antibiotics containing clavulanic acid might be an increasing source of adverse reactions because of specific IgE to clavulanic acid. More studies with a larger number of patients are needed to support these conclusions.

Our report focuses on 9 patients who were given diagnoses of allergy to clavulanic acid. The diagnosis was made on the basis of the skin test and basophil activation test results with the clavulanic acid molecule.

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Incidence of myeloproliferative hypereosinophilic syndrome in the United States and an estimate of all hypereosinophilic syndrome incidence

To the Editor:

Hypereosinophilic syndromes (HESs) include a heterogeneous group of diseases that have in common prolonged peripheral eosinophilia and organ damage in the absence of secondary causes for eosinophilia. Myeloproliferative (m-HES), lymphoproliferative, undefined, overlap, and associated variants of HES are now recognized,[1,2] and new therapeutic approaches to each are evolving.[3] Because no population-based data exist that address even such basic descriptive measures as incidence or prevalence, we examined a publicly available data source to evaluate the incidence of myeloproliferative HES/chronic eosinophilic leukemia and extrapolated from these data to estimate the incidence and prevalence of all HES.

The Surveillance, Epidemiology and End Results (SEER) program for cancer, funded by the National Cancer Institute, has been collecting data on HES since 2001 by using the International Classification of Diseases for Oncology, version 3, rubric of 9964/3 (HES including chronic eosinophilic leukemia), under the general category of chronic myeloproliferative disorders.[4] The authors used SEER∗stat software (http://www.seer.cancer.gov/seerstat version 6.4.4, Bethesda, Md, 2008) to calculate age-adjusted incidence in this group of patients; estimates from the literature were then used to extrapolate to the incidence of all HES.

Over the 5-year period (2001-2005), 131 incident cases in persons were reported in the SEER 17 area registries, resulting in a crude incidence of 0.035 per 100,000 and an age-adjusted rate of 0.036 per 100,000 person-years (95% CI, 0.030-0.042/100,000). There were 78 males and 53 females (age-adjusted rates, 0.044 and 0.027/100,000 person-years, respectively), and the male-to-female ratio was 1.47. Median age at diagnosis was 52.5 years, and rates increased with age to a peak in the range of 65 to 74 years (Fig 1).

One caveat in considering these data on m-HES is the intervening recognition of the etiology (and new diagnostic testing) for myeloproliferative forms of HES as a result of the Fip1-like 1–platelet-derived growth factor receptor α fusion gene *(FIP1LI-PDGFRA)* chromosomal fusion.[5] It is not recorded in the publicly available SEER data whether testing for *FIP1LI-PDGFRA* or other HES-related chromosomal fusions was done for the 131 patients. However, the majority had pathologic confirmation either through direct histologic examination (73%) or through a laboratory test or marker (15%), although no details are available in the public use database.

Does this age-adjusted incidence rate of 0.036 per 100,000 allow insight into the incidence of all HES? Gotlib and Cools[6] reviewed the proportion of *FIP1L1-PDGFRA*$^+$ individuals in 8 series of patients with HES and concluded, given the various selection biases in the data, the true value probably lay between 10% and 20%. If m-HES accounts for as little as 10% or as much as 20% of all HES, projecting from the SEER incidence rate for m-HES/chronic eosinophilic leukemia (CEL) would imply an annual age-adjusted incidence rate for all HES of between 0.18 per 100,000 and 0.36 per 100,000 person-years. These estimates have to be considered as upper bounds because they assume that all cases in the 9964/3 category are *FIP1L1-PDGFRA*$^+$. Unfortunately, the proportion of cases that are positive for the gene versus CEL and other m-HES cannot be determined from the SEER data,